TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

74

4. Budget Plan
for first year

Application For Research Grant

Overhead
Other

Date: March 11, 1955

Total

1. Name of Investigator: Cedilie Leuchtenberger, Ph.D.

2. Title: Associate Professor of Cytology, School of Medicine
Western Reserve University

3. Institution & Address: Institute of Pathology
Western Reserve University
Cleveland 6, Ohio
2085 Adalbert Road

4. Project or Subject: Quantitative Analysis of Nucleoproteins in Tissues from Animals Subjected to Tobacco Smoke by Microspectrophotometry and Interference Microscopy Correlated with Cytological and Histological Studies.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

A. Specific Aim. The purpose of this project is a simultaneous study of the cytochemical, cytological and histological alterations which may be induced in organs of animals which have been exposed to tobacco smoke.

Since the nucleoproteins constitute essential building stones of all cells in every tissue and are closely associated with their genetic properties, growth and metabolism, it is felt that an investigation of desoxyribose nucleic acid (DNA), ribose nucleic acid (RNA) and proteins in cells under the influence of tobacco smoke may reveal changes prior to their morphological manifestations.

The recent development of microspectrophotometric methods and interference microscopy which allow the quantitative estimation of nucleoproteins and dry mass determination in single cells in situ directly under the microscope make it possible to approach a study at such a sensitive level. Furthermore, previous studies by these methods on various normal and malignant tissues in both animals and humans (see enclosed reprints) have revealed that microspectrophotometry is indeed an excellent method for detecting submorphological changes.

Closely correlated with these quantitative cytochemical analyses will be detailed cytological and histological examinations of all the tissues concerned. In the cytological studies, carefub attention will be given to the possible occurrence of mitosis in otherwise resting tissues and also to any abnormalities which may occur in such cellular elements as nucleoli, nuclei and cytoplasm. In the histological studies, special emphasis will be given to possible hyperplastic and degenerative changes in the cardiovascular system, the respiratory system, the liver, kidneys, adrenals and reproductive organs.

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/s/ [illegible]
Business Officer of the Institution

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Source: https://www.industrydocuments.ucsf.edu/docs/zqm0000

#5 continued.

B. Materials and Methods. Mice of known genetic strains of both sexes at various ages will be exposed to tobacco smoke in a smoking machine similar to that of Essenberg (Science 116:561,1952) for varying intervals. Control animals of the same strain, sex and age will be put into a control chamber for the same lengths of time.

Both control and experimental animals will be kept on a stock diet, a diet rich in protein and a diet rich in fat and also subjected to various stresses (such as work on a treadmill).

Each group and controls will be sacrificed at varying intervals and tissues will be removed for cytochemical, cytological and histological examination.

For the quantitative estimation of the nucleoproteins, visible and ultraviolet microspectrophotometry will be employed. The validity of these microspectrophotometric methods has been established by comparing them with other standard methods (see enclosed reprints entitled "The Nucleoproteins in Cell Growth and Division" and "Critical Evaluation of Feulgen Microspectrophotometry for Estimating Amount of DNA in Cell Nuclei".)

For the determination of the mass of cells and cell parts, the interference microscopic method will be employed according to the technic of Davis and Wilkins (Nature 169:541,1952).

#9 ~~continued.~~ Additional Requirements

PERSONNEL

1.	Experienced Cytologist at Ph.D. level	$6000/yr. + 2% Soc. Sec. =	$6084
2.	Research technician trained in histological procedures and animal experimentation	3600 " " " "	3672
3.	Part time secretary and research librarian	1200 " " " "	1224
4.	Animal caretaker and glassware washer	2400 " " " "	2448
		Total	$13428

PERMANENT EQUIPMENT

1.	Interference microscope	(Approximate estimation)	$ 3000
2.	Smoking apparatus, treadmill, mazes	"	1500
3.	Animal cages	"	400
4.	Research microscope	"	1500
			$ 6400

EXPENDABLE SUPPLIES

1.	Animals and animal food	(Approximate estimation)	$ 500
2.	Special quartz slides, and other laboratory supplies (chemicals, glassware, dyes, enzymes, etc.)	"	800
			$ 1300

REPRINTS ENCLOSED

1. The Nucleoproteins in Cell Growth and Division, Cecilie Leuchtenberger, in Statistics and Mathematics in Biology, Ed. by Oscar Kempthorne, Theodore A. Bancroft, John W. Gowen, and Jay L. Lush. Iowa State College Press. Ames. 1954.

2. A Microspectrophotometric Study of the Desoxyribose Nucleic Acid Content in Cells of Normal and Malignant Human Tissues, C. Leuchtenberger, R. Leuchtenberger and A. M. Davis. Am. J. Path. 30, 65-85 (1954).

3. Critical Evaluation of Feulgen Microspectrophotometry for Estimating Amount of DNA in Cell Nuclei. C. Leuchtenberger. Science, 120, 1022-1023 (1954).

4. The Desoxypibosenucleic Acid Content in Spermatozoa of Fertile and Infertile Human Males. C. Leuchtenberger, F. Schrader, D. R. Weir and Doreen P. Gentile. Chromosoma 6, 61-78 (1953).

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Source: https://www.industrydocuments.ucsf.edu/docs/zgvm0000

6. Budget Plan:

for first year

Salaries	$13,428
Expendable Supplies	1,300
Permanent Equipment	6,400
Overhead	1,771124
Other	300
Total	$23,199.24

7. Anticipated Duration of Work:

Three years

8. Facilities and Staff Available: The facilities which are available for these studies is an establishe laboratory of cytochemistry (Head, Dr. C. Leuchtenberger) and suitable animal quarters in the Institute of Pathology at Western Reserve University. The cytochemical laboratories are well equipped for all tissue work, as well as for the analysis of intracellular sub-~~xxxx~~ stances. Some of the major items of permanent equipment are (1) Four microspectrophotometric apparatuses; (2) one ultraviolet microspectrophotometer; (3) five research microscopes; (4) one phase microscope; (5) one photomicrographic setup; (6) one Beckman spectophotometer; (7) one freeze-drying apparatus for the fixation and embedding of tissues.

There is also a team of workers (professional and technical) comprising cytochemists, pathologists and research assistants.

9. Additional Requirements:

See preceding page.

5. (Attach Plan of Procedure (Use reverse side if additional space is needed)

10. Additional Information (Including relation of work to other projects and other sources of supply):

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Signature /s/ Cecilie Leuchtenberger, Ph.D.
Director of Project

/s/ S. F. Agnew
Business Officer of the Institution

S. F. Agnew, [illegible]

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Source: https://www.industrydocuments.ucsf.edu/docs/zgvm0000